

Final Technical Report

**Light and Gravity Effects on Circadian Rhythms
of Rhesus Macaques**

NAG2-801

Charles Fuller
Section of Neurobiology, Physiology, & Behavior
University of California, Davis
Davis, California 95616-8519

APR 22 1997
C.A.S.I.

Introduction

Temporal integration of a biological organism's physiological, behavioral and biochemical systems depends upon its circadian timing system. The endogenous period of this timing system is typically synchronized to the 24-hour day by environmental cues.

The daily alternation of light and dark has long been known as one of the most potent environmental synchronizers influencing the circadian timing system. Alterations in the lighting environment (length or intensity of light exposure) can also affect the homeostatic state of the organism.

A series of experiments was performed using rhesus monkeys with the objective of defining the fundamental properties of the circadian rhythm of body temperature. Three major experiments were performed in addition to several preliminary studies. These experiments explored 1.) the response of the rhesus body temperature rhythm to varying day length and light intensity; 2.) the response of the body temperature rhythm to light exposure as a function of time of day; and 3.) the characteristics of the metabolic heat production rhythm which is responsible for the daily cycle in body temperature. Results of these three completed experiments will be reported here. In addition, preliminary experiments were also performed in social entrainment of rhesus circadian rhythms and the properties of rhesus body temperature rhythms in constant conditions, where no external time cues were provided.

Four adult male rhesus monkeys served as subjects in all experiments. All experiments were performed at the California Regional Primate Research Center. Each animal was implanted with a biotelemetry unit that measured deep body temperature. All surgeries were performed by a board certified veterinary surgeon under sterile conditions. The biotelemetry implants also provided an index of activity level in each animal. For metabolic heat production measurements, oxygen consumption and carbon dioxide production were measured and the caloric equivalent of these was calculated. Specific methodologies are described in detail below.

I. Response of rhesus monkeys to varying photoperiod

Rationale and specific aims

The circadian rhythm in body temperature is normally entrained to the 24 hour day by the daily alternation of light and dark, or light-dark cycle. While the 24 hour light-dark cycle is generated by the rotation of the earth about its axis, because of the tilt of the earth's axis, the relative length of the light (photoperiod) and dark (scotoperiod) portions of the 24 hour day are dependent upon latitude and the position of the earth relative to the sun. The photoperiod is commonly used as a cue for seasonal adjustments in physiology and behavior.

One physiological rhythm which is known to respond to changes in photoperiod in other mammals is the rhythm in body temperature. In diurnal, or day-active, species such as humans the elevation in body temperature corresponding to the light period is expected to be prolonged with greater photoperiod.

A second physical aspect of light that can affect circadian rhythms is intensity. In other species there is a direct response of body temperature to light intensity, with temperature being elevated at higher light levels.

These responses have not previously been studied in rhesus monkeys; the appropriateness of rhesus as a model for human rhythms would be better established by elucidation of the responses of their circadian rhythms to these aspects of the light-dark cycle. The first study we performed examined the effects of: 1.) length of the light portion of a 24 hour light-dark cycle (photoperiod) and 2.) light intensity on the circadian rhythms of body temperature and activity of the rhesus monkey.

Methods

Four adult male Rhesus Monkeys (*Macaca mulatta*) weighing 10 ± 1 kg were individually housed in cages at an ambient temperature of 25 ± 1 °C. White noise (70 ± 3 dB) was provided to mask external sounds which might provide time cues in addition to the light-dark cycle. Feeding times on consecutive days

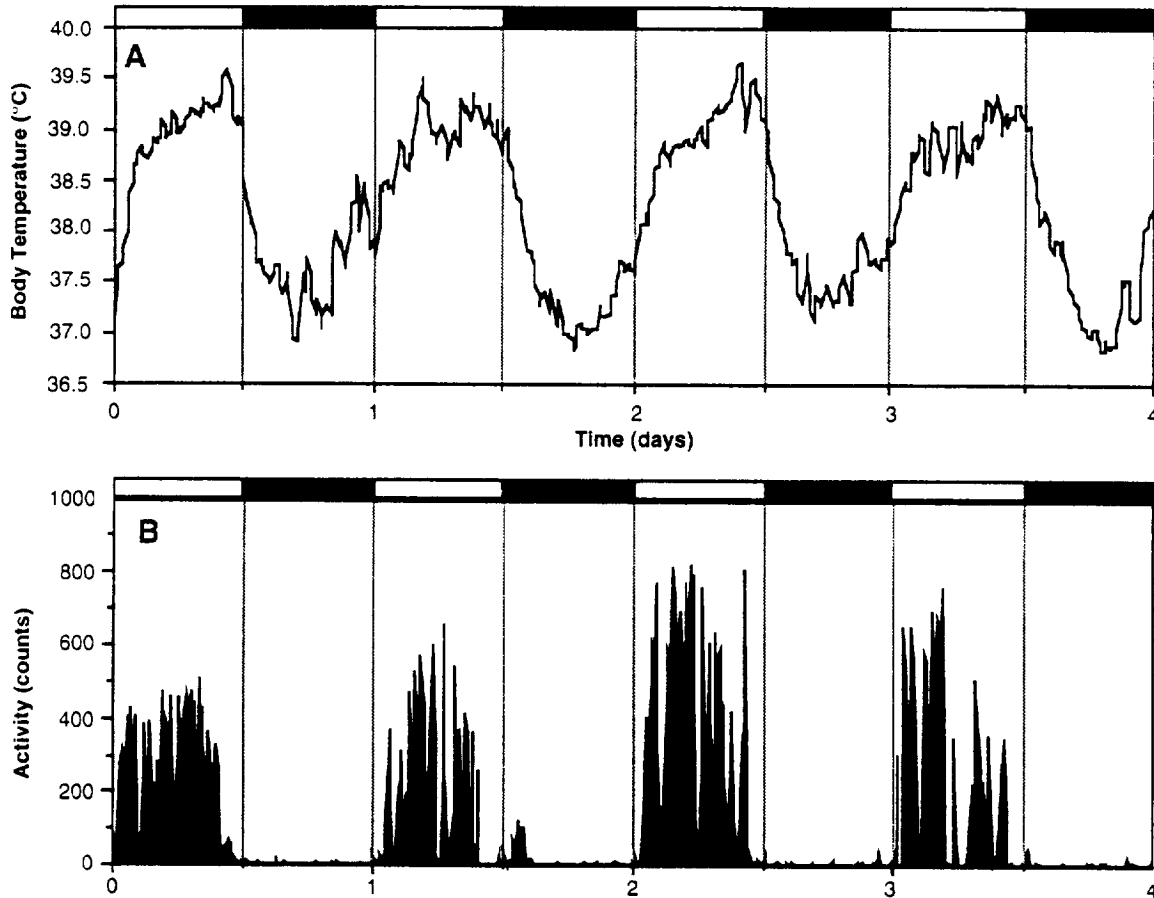
were alternated between morning and evening to prevent entrainment by feeding or social cues. Water was available *ad lib*.

Animals were exposed to 24 hour light-dark cycles with photoperiods of 12 hours (LD 12:12), 14 hours (LD 14:10) and 16 hours (LD 16:08); lights-on was always at 0600 PST. Light was provided by cool-white fluorescent bulbs. The light intensity was the average measured within the animal's cage. For each photoperiod two levels of light intensity were tested: 200 lux and 1500 lux. Animals were placed successively in LD 12:12, LD 14:10 and LD 16:08. In each photoperiod the animals were exposed for 9 days to a light intensity of 200 lux followed by 5 days at 1500 lux.

Data for body temperature and physical activity were obtained at 10 minute intervals by telemetry. The biotelemetry transmitter was implanted in a muscular pocket outside the parietal peritoneum. The transmitted data were collected using a microcomputer based data acquisition system (Data Sciences, Inc.). The raw data were averaged to a single cycle representing mean values for each time of day. Mean, amplitude and phase were determined by fitting a sine wave to successive 24-hour segments of the raw data. Two-way analysis of variance and Student's t-test were used to compare data.

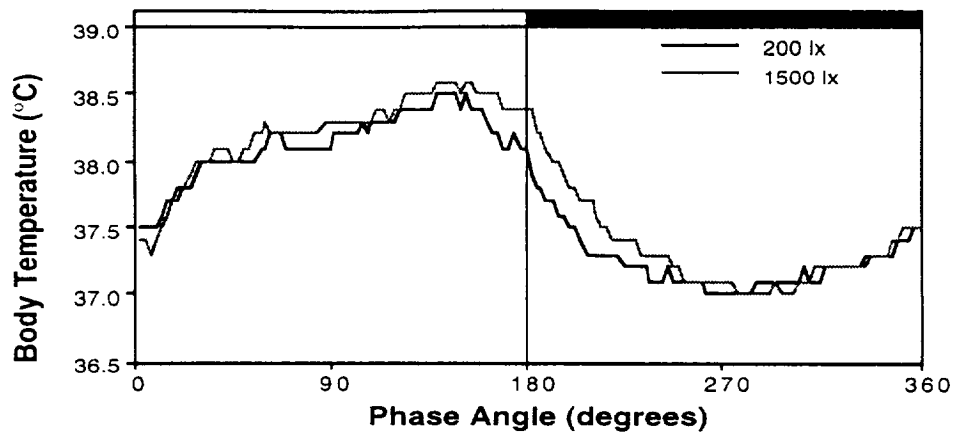
Results

In all photoperiods and light intensities both the temperature and physical activity rhythms were entrained to the 24 hour LD cycle. Both the length of the photoperiod and light intensity affected the rhythms of body temperature and activity.

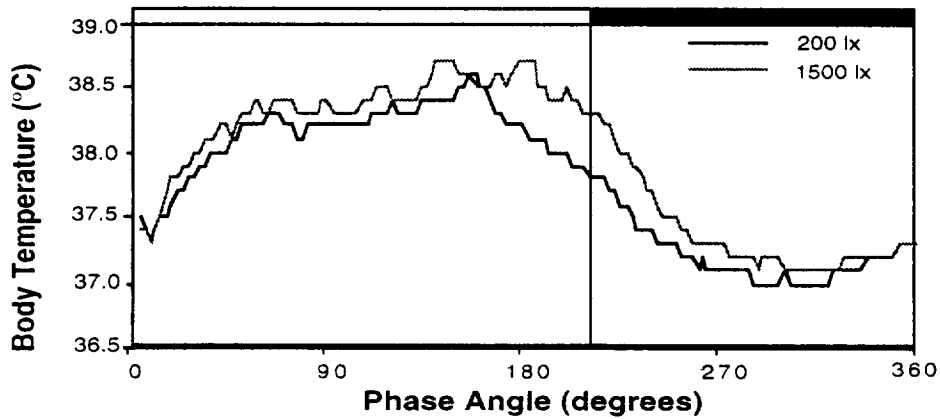


In figure I-1 (above), data are given for a representative monkey in LD 12:12 at 200 lux showing a 24 hour cycle of body temperature (upper panel) and physical activity (lower panel). Body temperature rises prior to light onset, shows a maximum in the middle of the light period, decreases prior to lights-off, and remains depressed during the dark period. Physical activity is almost entirely limited to the light period.

A LD 12:12



B LD 14:10



C LD16:08

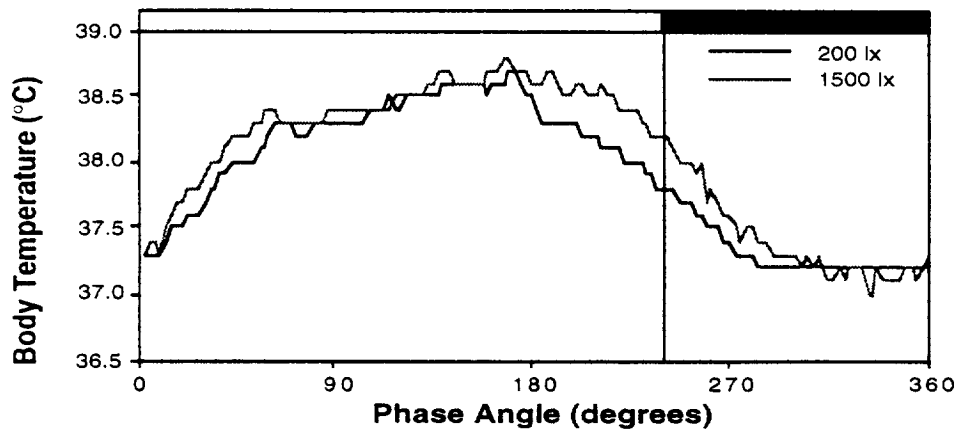
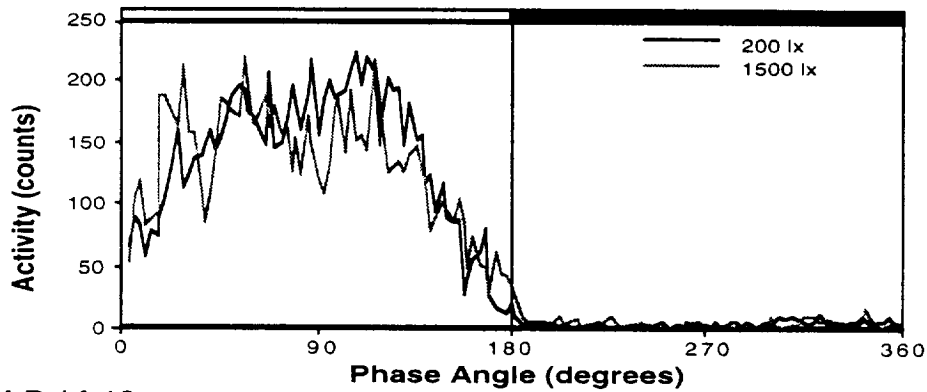


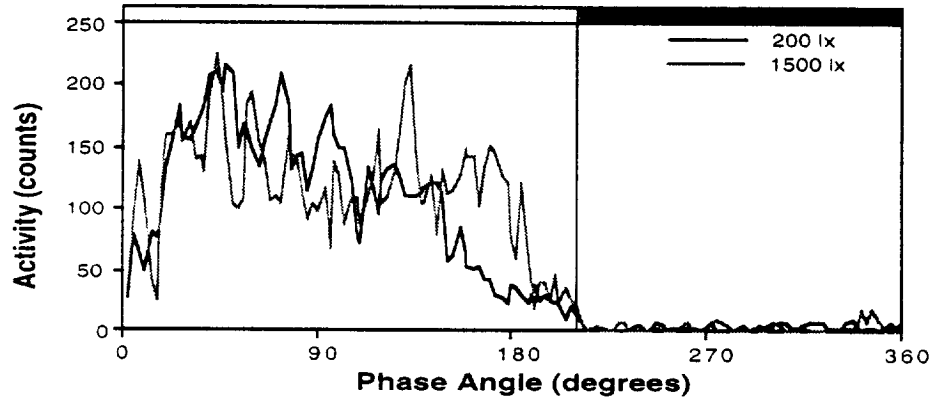
Figure I-2 (above) shows a characteristic waveform in each photoperiod and illumination level in plots of the average body temperature rhythm for all four animals at 200 and 1500 lux. (A, LD 12:12; B, LD 14:10; C, LD 16:08). The shape of the educed waveform varies according to the photoperiod. The initial rise in body temperature follows a similar time course for each photoperiod, but

remains elevated longer as the photoperiod lengthens. The direct effect of the light intensity is shown by increased body temperature in the higher light intensity

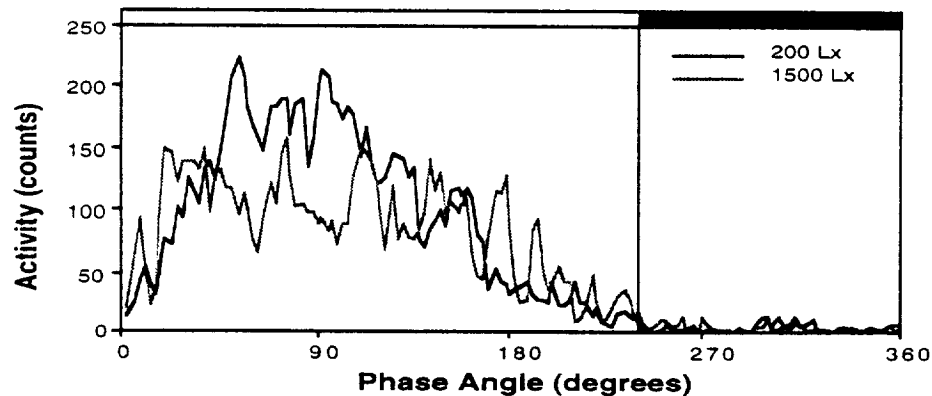
A LD 12:12



B LD 14:10

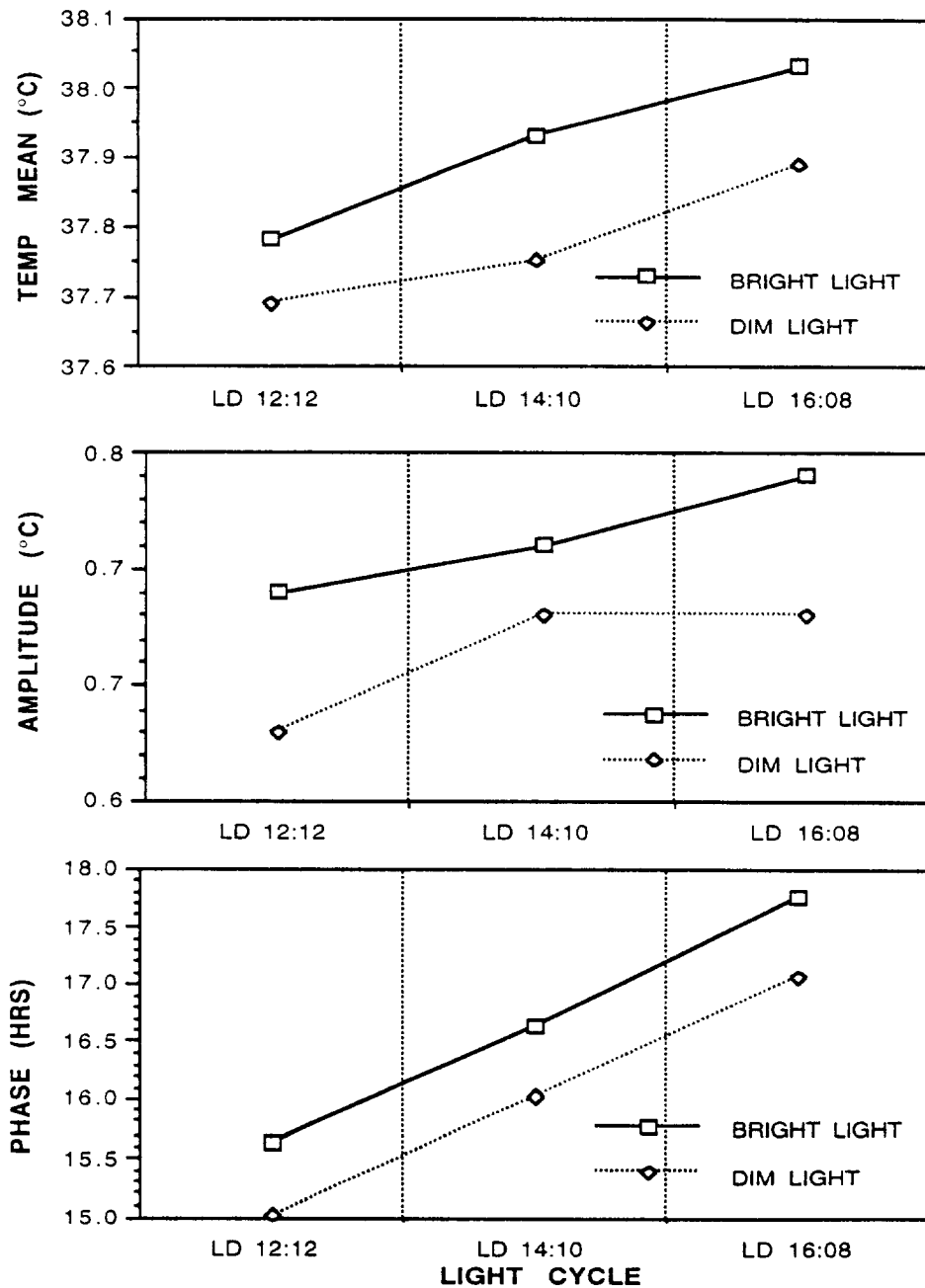


C LD 16:08



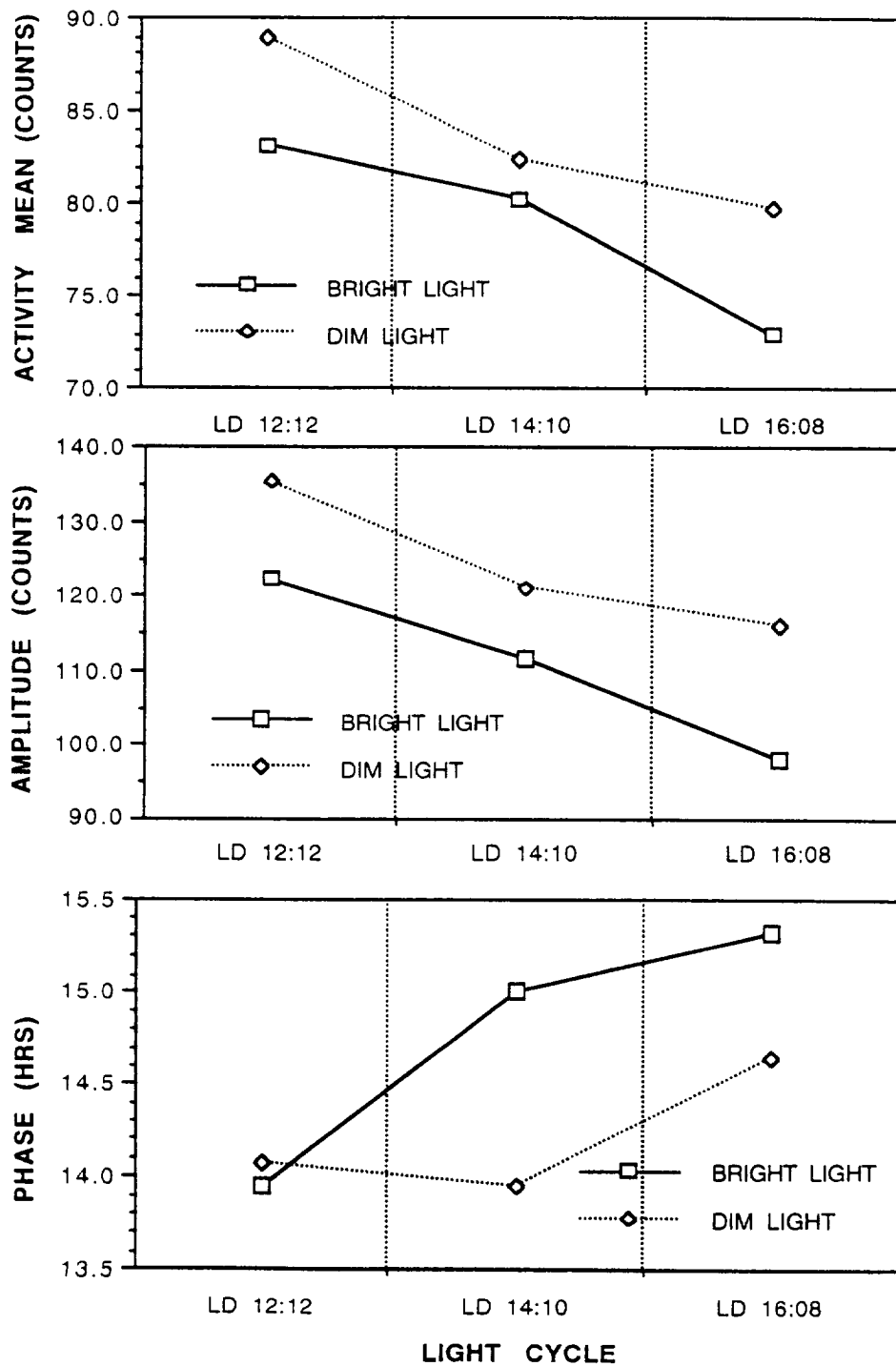
Similarly for activity, figure I-3 (above) shows a characteristic waveform in each photoperiod and illumination level in plots of the average physical activity rhythm for all four animals at 200 and 1500 lux. (A, LD 12:12; B, LD 14:10; C, LD

16:08). Physical activity shows a characteristic waveform according to photoperiod. Light intensity also affects the shape of the average wave form. Higher light level produces a phase delay and decreases activity levels.



Average properties of the body temperature rhythm, the mean, amplitude, and time of peak, or acrophase, are summarized in Fig. I-4 (above). Mean temperature (upper panel) increases with longer photoperiod and higher light

intensity. Amplitude of the temperature rhythm (middle panel) increases with the length of the photoperiod and light intensity ($p < 0.05$). The phase angle (lower panel) is delayed with increasing photoperiod ($p < 0.01$) and light intensity ($p < 0.01$).



The responses of activity level to photoperiod and light intensity were more variable than those of body temperature as is shown in figure I-5 (above). Mean activity (upper panel) decreases with increased photoperiod and is slightly lower at the higher light intensity. Amplitude of the activity rhythm (middle panel) decreases with increased photoperiod as well as increased light intensity. The phase of the activity rhythm (lower panel) is (at 200 lux) marginally advanced in LD 14:10 relative to LD 12:12 and delayed in LD 16:08. At 1500 lux the phase is consistently delayed by increased photoperiod ($p < 0.01$).

Summary and conclusions

In conclusion, this first experiment has shown that at least two different aspects of the lighting environment, intensity and photoperiod, affect the rhesus circadian timing system. Rhesus monkeys respond to changes in photoperiod and light intensity much like humans but quite differently from lower vertebrates. The similarities between the human and the rhesus offer unique advantages in the use of this animal model in the study of the circadian timing system.

II. Response of rhesus body temperature rhythms to light exposure as a function of time of day.

Rationale and specific aims

The first study demonstrated that alteration of the photoperiod within a 24 hour light-dark cycle not only modified the timing and waveform of circadian rhythms, but also the levels of body temperature and activity of the Rhesus monkey. However, such body temperature and activity shifts could have been produced by either the influence of light on the circadian timing system or directly on homeostatic mechanisms, those dedicated to maintaining relative constancy of body temperature within a limited range.

A second study was required to distinguish between the influence of the circadian timing system on temperature and activity rhythms from the direct effects of light on homeostatic systems. To separate the effects of light from those of the circadian system, a high frequency light-dark cycle (LD 2:2) was employed. This light-dark cycle provides light exposure at varying phases of the internal, endogenously generated, circadian cycle.

Methods

Four adult male Rhesus Monkeys weighing 9-11 kg, were used in this study. They were individually housed in cages placed in a room illuminated at 1500 lux, with a light cycle of LD 16:08, and an ambient temperature of $25 \pm 1^\circ\text{C}$. White noise (70 ± 3 dB) was provided to mask external sounds which might provide time cues in addition to the light-dark cycle. Monkeys were fed with a commercial diet (Purina monkey chow) once daily at alternating times of day. The animals were previously found to be unable to entrain to such a feeding schedule of alternating short and long feeding intervals. Water was available *ad libitum* throughout the experiment. The animals were exposed to a LD 2:2 light cycle for 48 hours and following a week in a light cycle of LD 16:08, were exposed to 48 hours of a DL 2:2. Deep body temperature and activity were recorded every 10 minutes via telemetry, using a microcomputer based data acquisition system (Data Sciences, Inc.). Mean values for each time of day

were reduced to an average cycle. Magnitude of changes in body temperature and activity in different photoperiods were determined.

Results

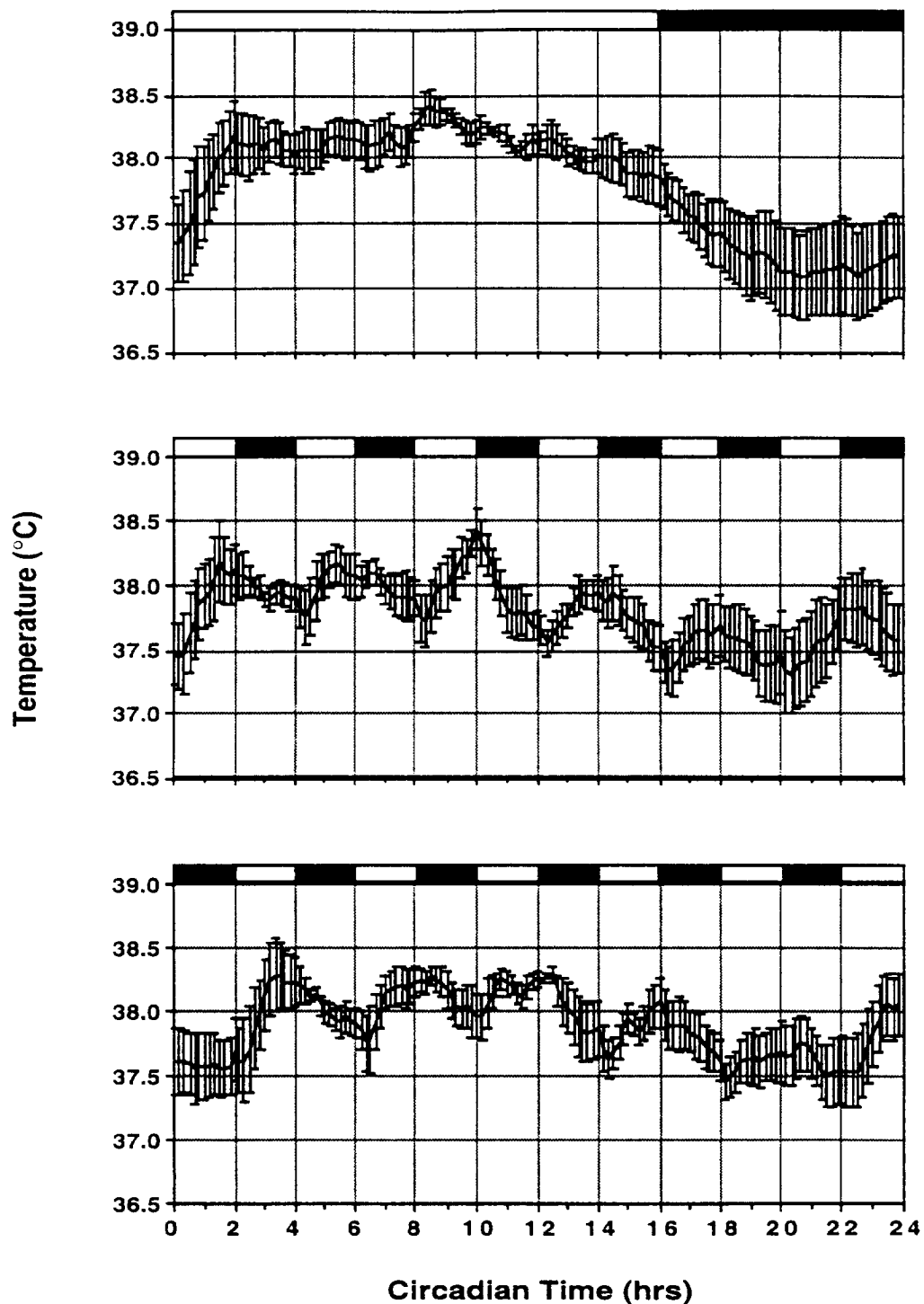
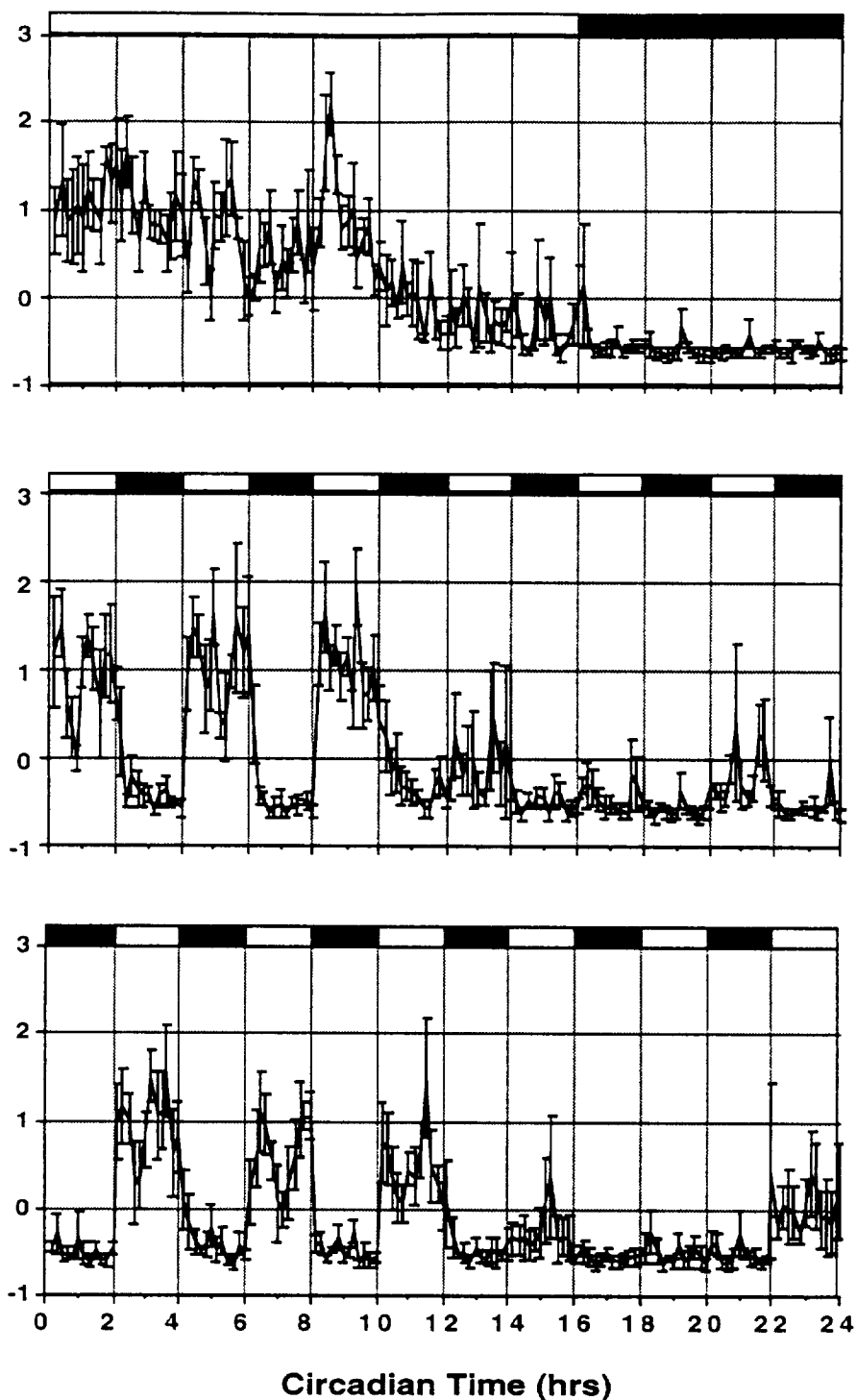
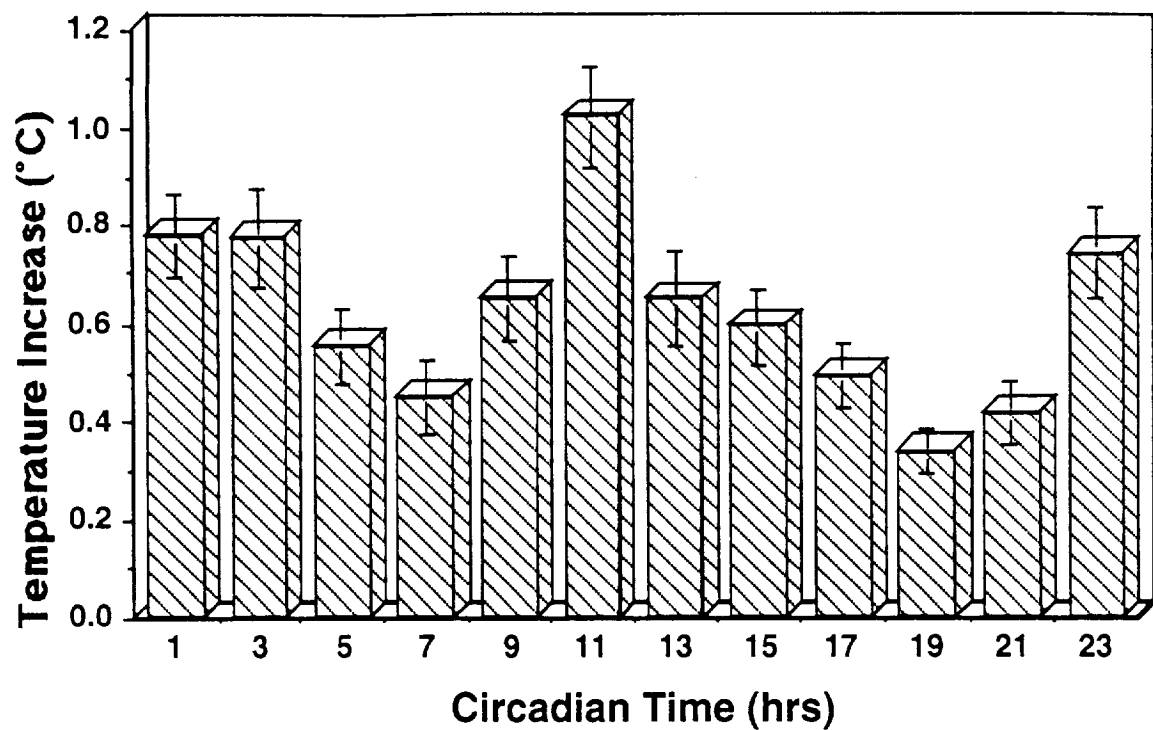


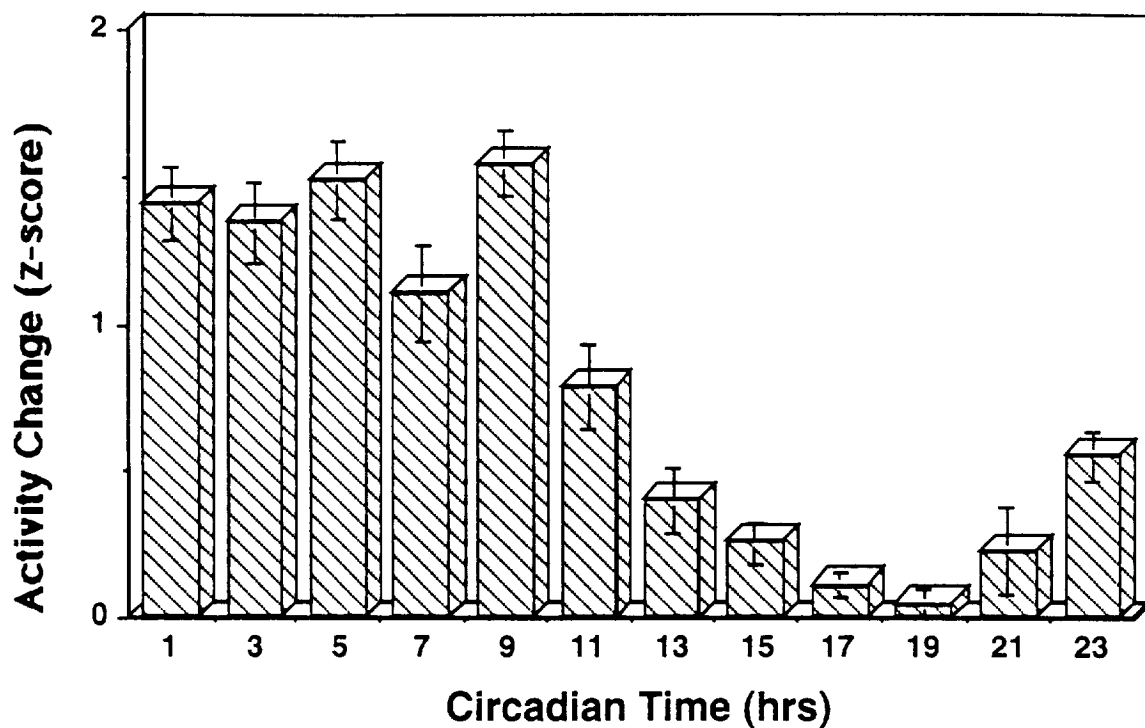
Figure II-1 (above) shows average body temperature (Mean $\pm$ SEM) waveforms for four Rhesus in LD 16:08 (upper), in LD 2:2 (middle), and in DL 2:2 (lower) light cycles. In all three panels, when considered at the same time of day, the mean body temperature is higher when the lights are on.



Corresponding waveforms for activity are shown in figure II-2. Shown here is mean activity (Mean $\pm$ SEM) waveforms for four Rhesus in LD 16:08 (upper), in LD 2:2 (middle), and in DL 2:2 (lower) light cycles. Activity, like temperature, is also higher when the lights are on.



The increase in body temperature (Mean + SEM) during the light periods of the LD 2:2 cycles is shown in figure II-3. In order to remove the influence of the underlying circadian rhythm of body temperature, temperature values from the corresponding 2 hour dark period were subtracted from the light values. This response is time of day dependent. The largest temperature response occurs during the light to dark transition period (dusk), with another smaller increase during the time of the dark to light transition (dawn).



Similarly, the light-induced increase in activity level is also time of day dependent, as shown in figure II-4. This figure shows activity (Mean + SEM) during light period of both LD and DL 2:2 as compared to the complementary dark period of the reverse LD cycle. However, in contrast to body temperature, the largest changes occur during the day and the smallest changes during the night.

Summary and conclusions

This experiment demonstrated that the regulation of both, body temperature and activity are directly influenced by light intensity. Further, this influence is time-of-day dependent. Although the trends are similar, the time-of-day responses are regulated differently for body temperature and physical activity. Thus, the normal 24 hour diurnal variation in body temperature and activity are the combined results of: 1) the entraining effects of light on the circadian system, 2) the circadian timing system influence on homeostatic mechanisms and, 3) the direct effects of light intensity on these homeostatic systems.

III. Metabolic heat production rhythm and its relation to the body temperature rhythm in rhesus monkeys.

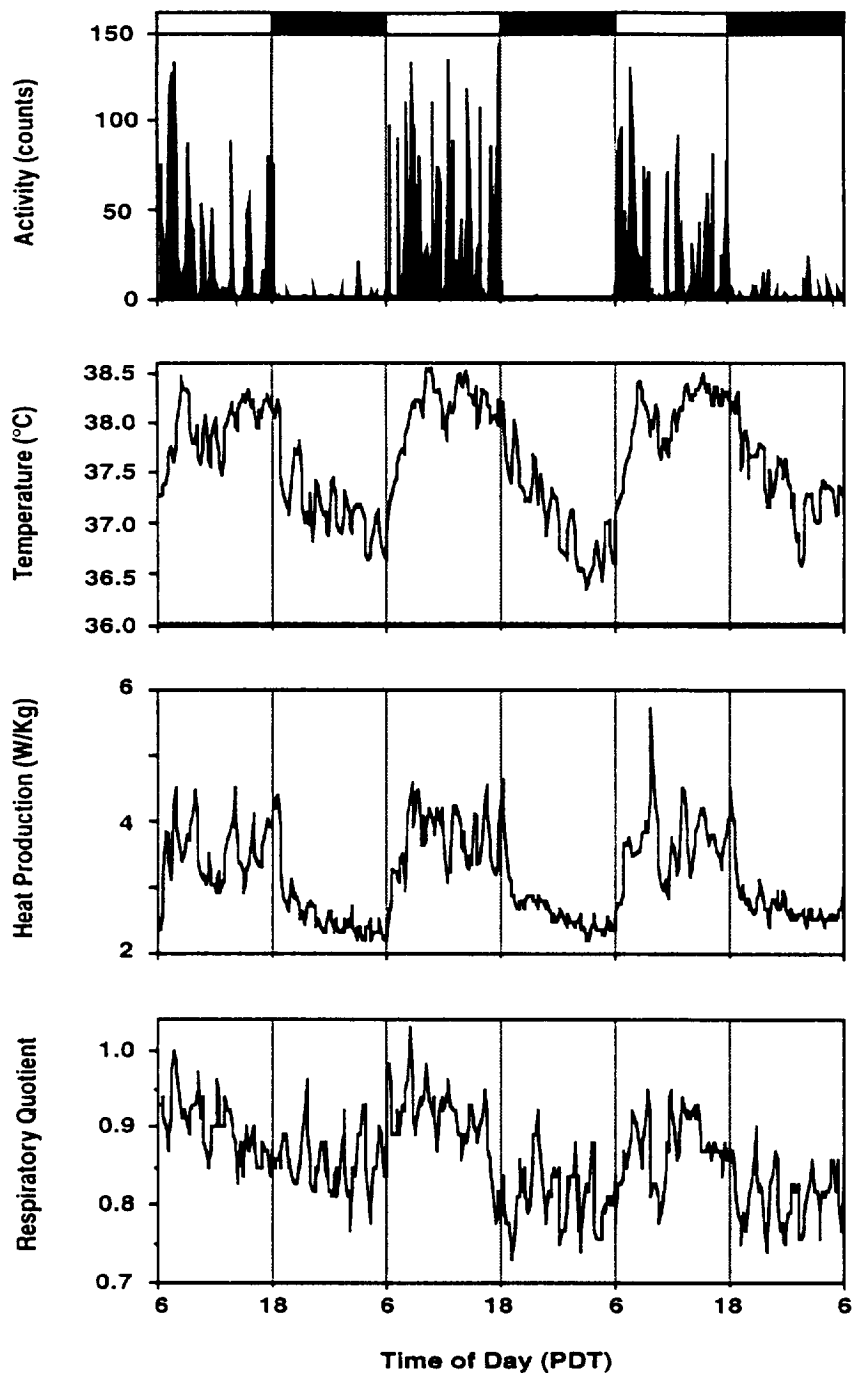
Rationale and specific aims

The body temperature rhythm, where temperature is elevated during the light portion of the day in diurnal species, implies that the regulation of body temperature will have different patterns during the day and night. The body temperature rhythm is determined by rhythms in both metabolic heat production and heat loss. Body temperature at any time of day depends on the balance between these rhythms and temperature regulation can be seen as a dynamic process. Heat production in primates depends mainly on muscle activity, although some other mammals predominantly utilize waste heat from metabolic processes. Heat dissipation may take several routes, including radiant and evaporative heat loss. This study examines the temporal relationship among rhythms of heat production, body temperature, and physical activity in the Rhesus monkey.

Methods

Four adult male Rhesus Monkeys (*Macaca mulatta*) weighing 10 ± 1 kg were individually housed. The cages were placed in closed chambers to provide constant air circulation at an ambient temperature of 25 ± 1 °C.. The animals were maintained in LD 12:12 (800 lx /0 lx) with food and water available *ad libitum*. Chambers were opened and food and water replenished as required at 2-3 day intervals between 8 and 10 AM. Data for body temperature and physical activity were obtained at 10 minute intervals by a radio transmitter implanted beneath the abdominal muscle adjacent to the peritoneum. The telemetry data were collected using a microcomputer based data acquisition system (Data Sciences, Inc.). Oxygen consumption and carbon dioxide production were measured every 10 minutes to determine metabolic heat production and to measure respiratory quotient (RQ). The raw data were averaged to a single cycle representing mean values for each time of day. Mean, amplitude and phase over 24 hours were determined for each rhythm by fitting a sine wave to successive 24-hour segments of the raw data.

Results



Data from a representative monkey are shown in figure III-1 (above; from top to bottom: physical activity, body temperature, heat production and respiratory quotient). Body temperature rose prior to light onset, with a maximum in the middle of the light period, decreased prior to lights-off, and remained depressed during the dark period. Physical activity was almost entirely limited to the light period. Heat production was elevated during the light period when both body

temperature and activity were higher. Respiratory quotient was elevated during the light period.

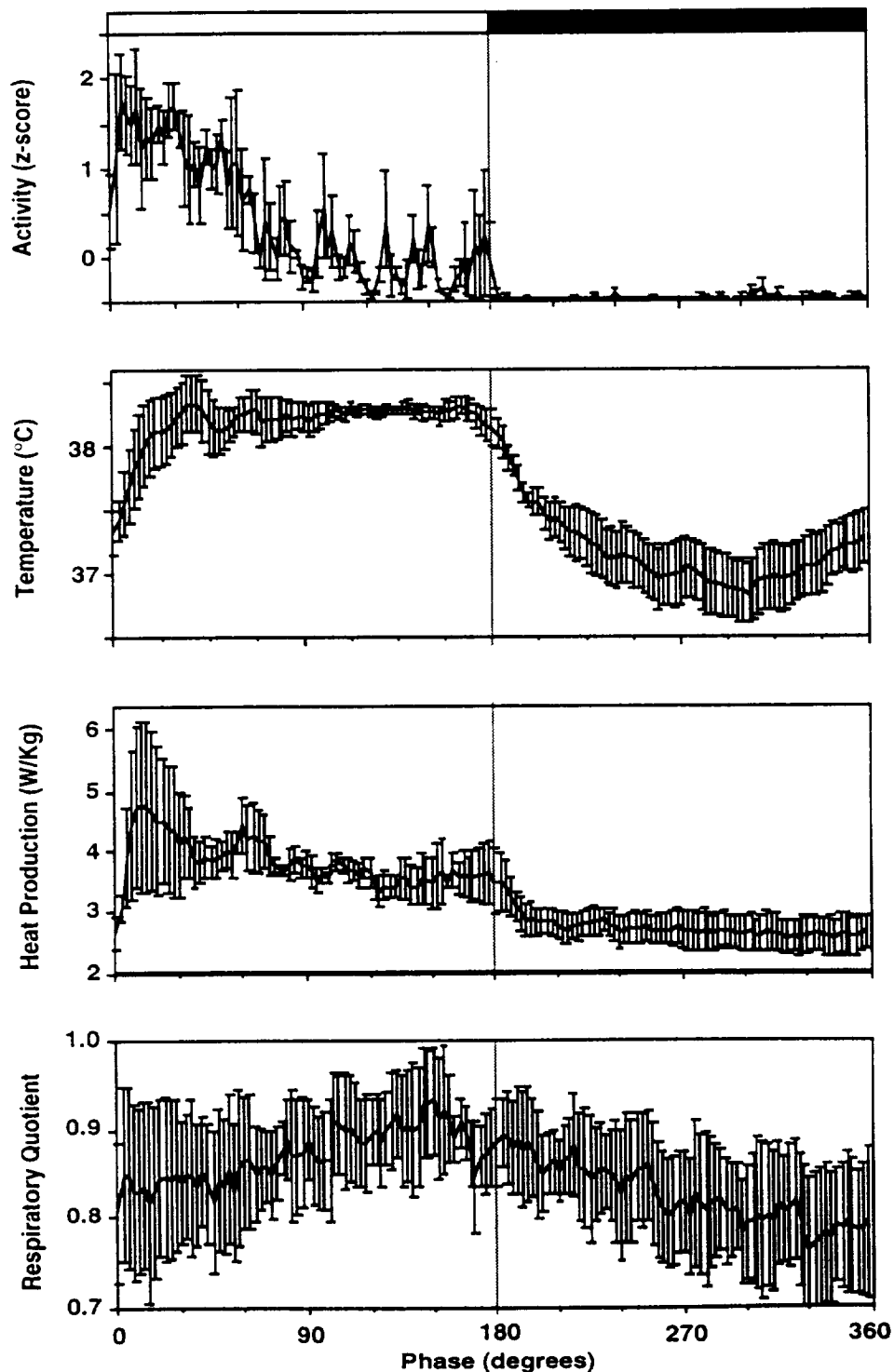
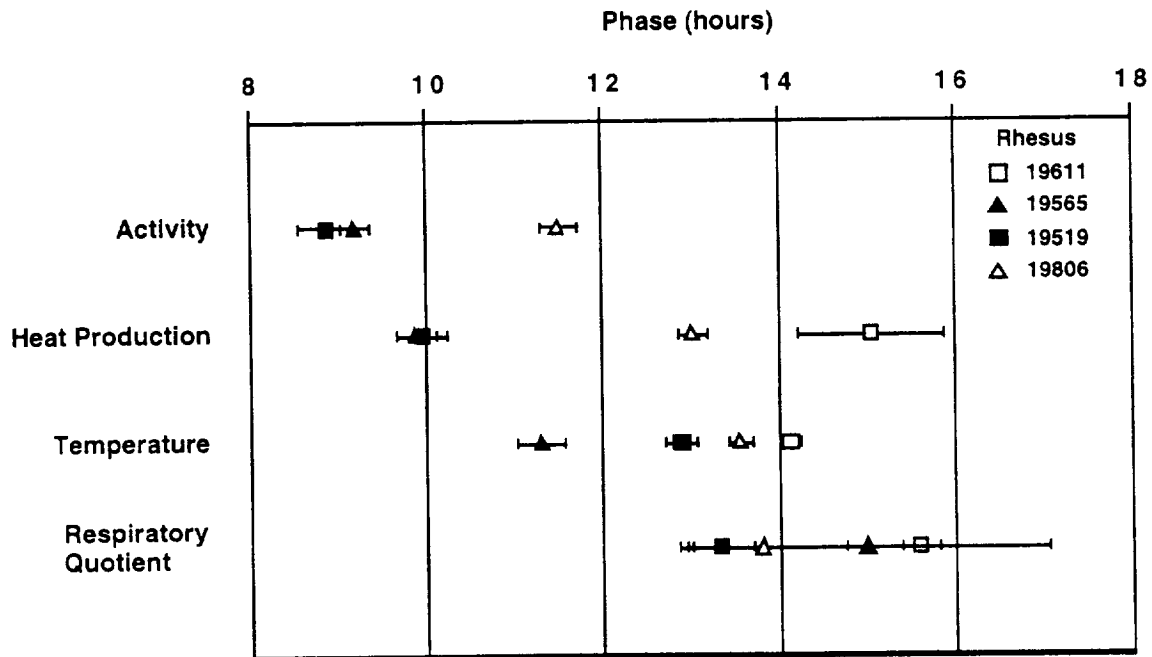


Figure III-2 (above) shows average waveforms ($\pm$ S.E.) over 24 hours (top to bottom: physical activity, body temperature, heat production and respiratory quotient). Body temperature, physical activity and heat production showed characteristic rhythms. Elevated activity after lights-on was followed by

increased metabolic rate which preceded the rise in body temperature. Body temperature remained elevated for most of the light period, while activity and heat production decreased. Thus the elevation in temperature must also involve the regulation of heat loss. Mean resting metabolism, during the dark period, was approximately that predicted by allometric equations. Respiratory quotient was more variable, but tended to increase during the animals' active period, when feeding occurred.



The average phase relationships ($\pm$ S.E.) among the circadian rhythms of activity, heat production, body temperature and respiratory quotient are summarized in figure III-3 (above). Each rhythm is summarized by the time of its peak as determined by a sine wave fit. Peak heat production lagged behind peak activity by approximately an hour (NS). Peak body temperature was more variable and occurred 2 to 4 hours after peak activity ($p < .05$). Temperature typically peaked after metabolic heat production. Respiratory quotient was more variable, but followed peak activity, metabolic heat production and body temperature.

Summary and conclusions

Activity, heat production, and body temperature showed daily rhythms with activity and heat production peaking earlier in the day than body temperature.

Increased activity and heat production early in the day may not be sufficient to explain the elevation of body temperature which persists throughout the animals' light period. Autonomic regulation of heat loss would also be expected to have a role in producing the daily temperature rhythm. Heat production during the inactive period was consistent with predicted metabolic activity based on the body mass. A daily rhythm in RQ was shown, consistent with diurnal feeding and nocturnal fasting. Individual variation in timing suggested that individual Rhesus, like humans, can be either 'early' or 'late' relative to the daily light-dark cycle.